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SPECIAL REPORT

Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a Pertussis toxin-sensitive mechanism

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Cannabinoid receptor ligands irreversibly inhibited peak voltage-activated Ca currents (44%) in NG108-15 cells; this inhibition was Pertussis toxin-sensitive. Inhibition was largely due to a reduction in the ω-conotoxin sensitive portion of high-voltage activated (HVA) current, although there was also a significant decrease in low-voltage activated current (56%) and in the nifedipine-sensitive portion of HVA current (41%).

Keywords: Cannabinoid; G-protein; Pertussis toxin; calcium current; neuroblastoma

Introduction Cannabinoids have a wide range of central actions (Howlett *et al.*, 1990), but their action at the cellular electrophysiological level is still unclear. A stereoselective receptor has been identified in brain, the activation of which causes inhibition of adenylyl cyclase (Howlett *et al.*, 1990). A cDNA for an appropriate G-protein coupled receptor has recently been cloned (Matsuda *et al.*, 1990). NG108-15 neuroblastoma x glioma hybrid cells also express this receptor (Matsuda *et al.*, 1990). In these cells, other receptors which couple negatively to adenylyl cyclase inhibit a voltage-gated calcium current (I_{Ca}) (e.g. α_2-adrenergic: Docherty & McFadzean, 1989; m2 and m4 muscarinic: Higashida *et al.*, 1990). We have therefore tested whether the cannabinoid receptor might also inhibit I_{Ca} in NG108-15 cells.

Methods I_{Ca} was recorded from chemically-differentiated NG108-15 cells as described by Caulfield *et al.* (1992). Whole-cell voltage-clamp recordings were made by use of a switching amplifier (Axon Instruments) operating at a sampling frequency of 4–10 kHz. Patch electrodes (3–5 MΩ) were filled with a solution containing (mM): CsCl 110, HEPES 40, EGTA 3, $MgCl_2$ 3, pH 7.4 with CsOH. Cells were superfused (5–10 ml min^{-1}) at 18°C–24°C with a modified Krebs solution containing (mM): tetraethylammonium chloride 120, KCl 3, $MgCl_2$ 1.2, HEPES 5, $NaHCO_3$ 23, $CaCl_2$ 2.5, D-glucose 11 and tetrodotoxin 0.5 µM (pH 7.4 when bubbled with 95% O_2: 5% CO_2). Cells were voltage-clamped at −90 mV and I_{Ca} evoked every 30 s by stepping for 500 ms to command potentials (V_c) in the range −100 mV to +60 mV. Current amplitudes were measured at peak or at the end of the voltage pulse, after leak subtraction (Caulfield *et al.*, 1992).

Acetylcholine chloride (ACh), nifedipine and Δ^9-tetrahydrocannabinol (THC) were purchased from Sigma Chemicals. *Bordatella pertussis* toxin was purchased from Porton International. CP55940 ([1α,2β(R)5α)-(-)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)-cyclohexyl] phenol) was a gift from Pfizer Central Research. Synthetic ω-conotoxin GVIA (CgTx) was a generous gift of Dr A.J. Cross (Astra Neuroscience Research Unit, London).

Results The cannabinoid ligands THC (1–30 µM) and CP55940 (10 nM–1 µM) produced slowly-developing inhibition of I_{Ca}, which maximized after 2–15 min (Figure 1a). The maximum inhibition achieved was independent of drug concentration and inhibition did not recover detectably after up to 45 min washing ($n = 3$). This lack of reversibility precluded determination of meaningful concentration – response relationships. Subsequent studies were carried out with fixed concentrations of THC (30 µM) and CP55940 (1 µM). Cannabinoid ligands inhibited both peak (40% ± 6%; Figure 2a) and end-of-pulse current at $V_c = 0$ mV (47% ± 12%). Canna-

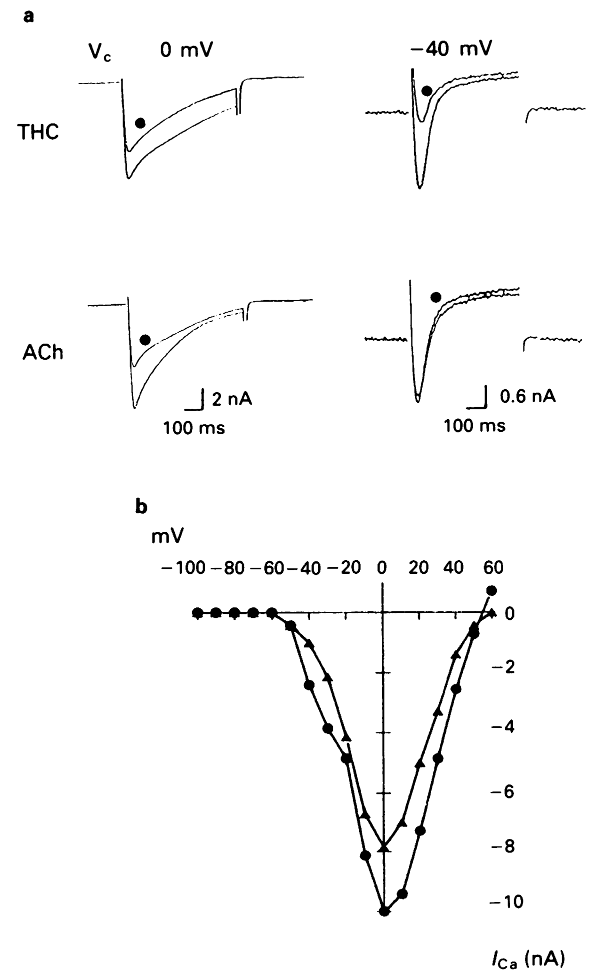

Figure 1 (a) Superimposed calcium currents are shown before and during (●) application of Δ^9-tetrahydrocannabinol (30 µM, THC) or acetylcholine (ACh, 30 µM). High voltage activated currents (V_c = 0 mV) are shown in the left panel and low voltage activated currents (V_c = −40 mV) are in the right panel. Holding potential −90 mV. (b) Leak-subtracted current-voltage relationships from the same experiment as (a) for control peak calcium currents (●) and currents in the presence of 30 µM THC (▲).

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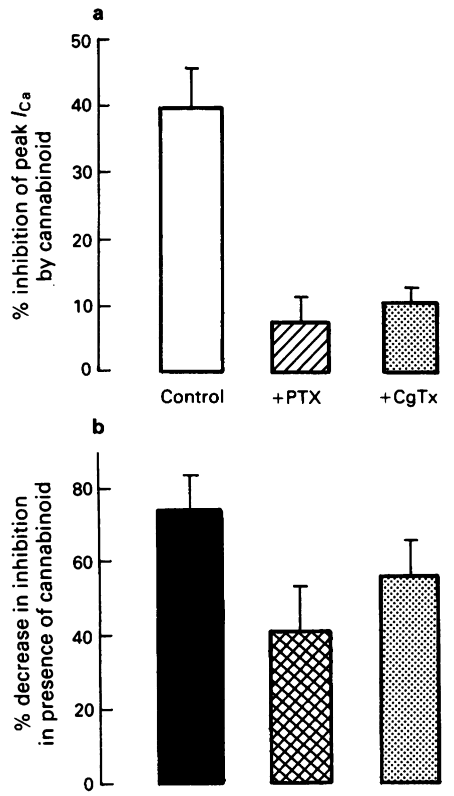

Figure 2 Mean inhibition of peak Ca current ($V_c = 0$ mV) by either 30 μM Δ^9-tetrahydrocannabinol (THC) or 1 μM CP55940 ($n = 10$; control) and after pretreatment of cells with 500 ng ml^{-1} Pertussis toxin (PTX; $n = 5$) or in the presence of 100 nM ω-conotoxin GVIA (CgTx; control inhibition 36% ± 10%, $n = 5$). Bars show s.e.mean. (b) Control inhibitions of peak I_{Ca} at $V_c = 0$ mV were: ACh (30 μM) 44% ± 14%; nifedipine (Nifed; 1–2 μM) 28% ± 6%; at $V_c = -20$ mV, $NiCl_2$ (Ni^{2+}; 20 μM) was 50% ± 7%. Histograms show the percentage reduction of the inhibitory effect of these agents in the presence of either THC (30 μM) or CP55940 (1 μM). Bars show s.e.mean.

binoid-sensitive currents showed a peak at -3 mV ± 2 mV ($n = 10$; Figure 1b), but there was also significant inhibition of I_{Ca} at negative potentials (e.g. at $V_c = -40$ mV there was 24% ± 11% inhibition; $n = 8$).

The inhibitory effect of cannabinoids was reduced to 8% ± 6% at $V_c = 0$ mV (Figure 2a) after pretreatment of cells with Pertussis toxin ($n = 5$; 500 ng ml^{-1}; 8–48 h); in these cells, cannabinoid inhibition of peak I_{Ca} at $V_c = -40$ mV was 7% ± 6%, and inhibition of end-of-pulse current at $V_c = 0$ mV was 8% ± 6%. I_{Ca} in NG108-15 cells can be subdivided pharmacologically into a low-voltage activated (LVA) component, selectively blocked by Ni^{2+}, and two high-voltage activated (HVA) components inhibited by CgTx and nifedipine respectively (Caulfield *et al.*, 1992). In the presence of CgTx, the cannabinoid-sensitive current was reduced to 7% ± 3% (Figure 2a). The inhibitory action of ACh is restricted to the CgTx-sensitive component (Caulfield *et al.*, 1992) and as expected from this, pre-application of cannabinoids largely occluded the inhibitory effect of ACh (Figure 2b). However, unlike ACh (see Caulfield *et al.*, 1992), cannabinoids also reduced the inhibitory effects of nifedipine and Ni^{2+} (Figure 2b).

Discussion In common with other neurotransmitter agonists which inhibit adenylyl cyclase (see MacFadzean & Docherty, 1989; Higashida *et al.*, 1990), the cannabinoid receptor ligands THC and CP55940 inhibit I_{Ca} in NG108-15 cells in a Pertussis toxin-sensitive manner. However, the precedents from studies with other neurotransmitters suggest that G_o, rather than G_i, might be the G-protein responsible for I_{Ca} inhibition (McFadzean *et al.*, 1989). Further, the low potency of THC and CP55940 as inhibitors of I_{Ca} relative to their potency in inhibiting cyclase (see Howlett *et al.*, 1990) implies less efficient coupling of cannabinoid receptors to the G-protein mediating I_{Ca} inhibition than to G_i.

Although ACh inhibition was greatly reduced by cannabinoids, indicating considerable overlap of action, there were nevertheless significant differences between ACh inhibition and cannabinoid inhibition of I_{Ca}. Thus, not only did the cannabinoid inhibition not reverse, but cannabinoids also significantly inhibited both end-of-pulse HVA current and LVA current. This latter effect is relatively unusual for G-protein coupled receptors (see review by Scott *et al.*, 1991). Also, some residual inhibitory effect of cannabinoids persisted in the presence of CgTx, and (unlike ACh) the cannabinoids produced significant inhibition (about 40%) of the nifedipine-sensitive portion of peak HVA current and Ni^{2+}-sensitive LVA current. The inhibition of multiple components of Ca current by cannabinoids may imply the existence of multiple receptor subtypes, each inhibiting a different current component, or possibly coupling of one receptor type to more than one type of G-protein (there are two subtypes of G_o in NG108-15 cells; Mullaney & Milligan, 1990).

Notwithstanding this apparent complexity, inhibition of I_{Ca} might provide a cellular electrophysiological basis for some of the central actions of cannabinoids. It would therefore be interesting to know whether similar effects occur in central neurones and if so, whether they result in significant changes in neuronal excitability or transmitter release.

Similar findings were reported in preliminary form by Mackie & Hille (1991, *Soc. Neurosci. Abstr.*, **21,** 435.6) while this work was in progress (Mackie & Hill, 1992; *Proc. Natl. Acad. Sci. U.S.A.*, in press).

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